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The Autoimmunity-Associated Gene *PTPN22* Potentiates Toll-like Receptor-Driven, Type 1 Interferon-Dependent Immunity

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SUMMARY

Immune cells sense microbial products through Toll-like receptors (TLR), which trigger host defense responses including type 1 interferons (IFNs) secretion. A coding polymorphism in the protein tyrosine phosphatase nonreceptor type 22 (*PTPN22*) gene is a susceptibility allele for human autoimmune and infectious disease. We report that Ptpn22 selectively regulated type 1 IFN production after TLR engagement in myeloid cells. Ptpn22 promoted host antiviral responses and was critical for TLR agonist-induced, type 1 IFN-dependent suppression of inflammation in colitis and arthritis. PTPN22 directly associated with TNF receptor-associated factor 3 (TRAF3) and promotes TRAF3 lysine 63-linked ubiquitination. The disease-associated PTPN22W variant failed to promote TRAF3 ubiquitination, type 1 IFN upregulation, and type 1 IFN-dependent suppression of arthritis. The findings establish a candidate innate immune mechanism of action for a human autoimmunity “risk” gene in the regulation of host defense and inflammation.

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SUPPLEMENTAL INFORMATION
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INTRODUCTION

Early immune responses to viral and bacterial infection are critically dependent on cells of the myeloid lineage, including dendritic cells (DCs) and macrophages (Akira et al., 2006). Upon recognizing pathogen-derived products, these “innate” immune cells engage multiple processes that mediate host protection, including myeloid cell activation, recruitment of effector cells to sites of infection, and initiation of T and B lymphocyte functions. Innate immune cells promote these processes in part through elaboration of type 1 interferons (IFNs), including IFN-β and members of the IFN-α family (Takeuchi and Akira, 2010). Type 1 IFNs signal through IFN-α receptor (IFNAR) to induce upregulation of numerous genes that activate both innate and adaptive host immunity (Stetson and Medzhitov, 2006). Whereas type 1 IFNs are critical for host defense against infection, it is clear that they can also protect tissue by dampening the effects of proinflammatory factors in diverse disorders (González-Navajas et al., 2012; Kalliolias and Ivashkiv, 2010).

Pattern recognition receptors (PRRs) that sense conserved molecular motifs in pathogen products are principal inducers of type 1 IFNs. Type 1 IFN-inducing members of the Toll-like receptor (TLR) family of PRRs are located either in endosomes (TLR3, TLR7, TLR8, TLR9) or at the plasma membrane (TLR4) (Takeuchi and Akira, 2010). Upstream of type 1 IFN promoters, TLR signals activate transcription factors of the interferon response factor (IRF) family. TLR3 and TLR4 require function of IRF3, whereas TLR7 and TLR9 primarily act through IRF7 to induce type 1 IFNs (Takeuchi and Akira, 2010). Nondegradative K63-linked polyubiquitination of the E3 ubiquitin ligase TNF receptor-associated factor 3 (TRAF3) is also required for IRF activation and type 1 IFN induction after TLR ligation (Häcker et al., 2011). The mechanism whereby K63-linked poly-ubiquitination of TRAF3 is activated by TLR signals remains incompletely understood.

A single nucleotide polymorphism (rs2476601) in the *PTPN22* gene is strongly associated with increased risk of human autoimmune and infectious disorders (Rhee and Veillette, 2012). *PTPN22* is expressed widely in hematopoietic cell lineages (Arimura and Yagi, 2010). Human *PTPN22* encodes lymphoid phosphatase (Lyp), an intracellular tyrosine phosphatase (Cohen et al., 1999). Murine *Ptpn22* encodes the Lyp ortholog, PEST-domain enriched phosphatase (Pep) (Cloutier and Veillette, 1996). The major human *PTPN22* allele (1858C) codes for arginine at amino acid 620 position in PTPN22 (“PTPN22R”) (Gregersen et al., 2006). The disease susceptibility allele, 1858T, encodes tryptophan-620 (“PTPN22W”).

Overexpression experiments and studies in *Ptpn22*$^{-/-}$ T cells strongly suggested that both PTPN22 in humans and Ptpn22 in mice serve as negative regulators of T cell receptor signaling (Hasegawa et al., 2004). Variant PTPN22W differentially regulates human lymphocyte antigen receptor activation thresholds (Rhee and Veillette, 2012). However, the mechanisms by which altered PTPN22W function in lymphocytes might contribute to diverse PTPN22-associated diseases remain incompletely defined. Little has been reported concerning *PTPN22* function in nonlymphocytes. Zhang et al. reported hyperresponsiveness among DCs harboring a knocked-in Pep variant that is orthologous to human PTPN22W (Zhang et al., 2011). However, no innate immune cell function has yet been established for endogenous *Ptpn22* or for the human disease-associated variant PTPN22W.

Here, we report that *Ptpn22* connects TLR signaling in innate immune cells with the induction of type 1 IFN-driven host defense and immunoregulatory responses. We observed that *Ptpn22* positively regulated type 1 IFN production after TLR ligation in myeloid cells. Moreover, *Ptpn22* promoted type 1 IFN-dependent processes in vivo, including innate and adaptive cellular responses to viral infection, promotion of gut homeostasis, and suppression

of inflammatory arthritis. PTPN22 bound to TRAF3 and positively regulated TLR-induced TRAF3 lysine 63 (K63)-linked polyubiquitination. Finally, we report that human disease-associated variant PTPN22W exhibited reduced function with respect to TLR signaling, type 1 IFN production, and immunoregulation.

RESULTS

Ptpn22 Positively Regulates TLR-Driven Type 1 Interferon Production in Myeloid Cells

In macrophages and DCs, TLRs engage bifurcating signaling cascades that culminate in upregulation of type 1 IFNs and proinflammatory cytokines (Takeuchi and Akira, 2010). To address whether *Ptpn22* regulates TLR signaling, we stimulated bone marrow-derived macrophages (BMM) with lipopolysaccharide (LPS), a TLR4 agonist (Akira et al., 2006). We observed impaired induction of *Ifnb1* and *Ifna4* messenger RNA (mRNA) in *Ptpn22*$^{-/-}$ BMM (Hasegawa et al., 2004) (Figure 1A; see also Figure S1A available online). Notably, we found no defects in TLR4-induced upregulation of *Tnf*, *Il1b*, or other proinflammatory cytokine transcripts known to be dependent upon NF-κB activation, in *Ptpn22*$^{-/-}$ BMM (Figure 1A; Figure S1B). Similar to results in *Ptpn22*$^{-/-}$ macrophages, we observed impaired TLR4 agonist-stimulated upregulation of type 1 IFNs, but not of proinflammatory cytokines, in *Ptpn22*$^{-/-}$ bone marrow-derived DCs (BMDCs; Figure 1B; Figures S1D and S1E), and in RAW 264.7 cells, BMM, and human blood monocyte-derived macrophages (MDM) subjected to knockdown of *Ptpn22* or *PTPN22* with cell-permeable antisense oligonucleotide (ASO) (Figure 1C; Figures S1H, S1I, S1K, and S1N). Moreover, we found diminished IFN-α, but normal TNF production after stimulation of *Ptpn22*$^{-/-}$ Flt3 ligand-induced BMDCs (Flt3L-BMDCs) with polyU or CpG (TLR7 or TLR9 ligand, respectively; Figure S1G). We observed markedly decreased IFN-β production after stimulation of *Ptpn22*$^{-/-}$ BMM with LPS or the TLR3 agonist poly(I:C) (Figure 1D). Interestingly, we found impaired *Ifnb1* induction in *Ptpn22*$^{-/-}$ BMM transfected with poly(I:C) (Figure S1O), which stimulates type 1 IFN upregulation through engagement of MDA5, an intracellular RNA receptor (Kato et al., 2006). Together, these data indicated that Ptpn22 positively regulates type 1 IFN induction signaled by a range of PRRs expressed in multiple human and murine myeloid cell types in vitro. Loss of Ptpn22 has no effect on induction of proinflammatory cytokines, strongly suggesting that Ptpn22 selectively promotes subsets of PRR signals.

Intraperitoneal administration of TLR agonists results in rapid upregulation of type 1 IFNs and proinflammatory cytokines by liver-resident myeloid cells (Seki et al., 2000). Upon injection of LPS or poly(I:C) into *Ptpn22*$^{-/-}$ mice, we observed marked defects in hepatic *Ifnb1* and *Ifna4* mRNA upregulation (Figure 1E; Figure S1P), as well as reduced serum concentrations of IFN-β (Figure 1F). However, serum TNF and interleukin-6 (IL-6) concentrations increased normally after LPS injection (Figure S1Q). These data indicated that *Ptpn22* selectively potentiates TLR-induced type 1 IFN production in vivo.

We asked whether diminished TLR-driven type 1 IFN production by *Ptpn22*$^{-/-}$ myeloid cells and animals might result in defective type 1 IFN-dependent responses. We found that *Ptpn22*$^{-/-}$ BMM and BMDCs or *PTPN22* knockdown MDM stimulated with either LPS or poly(I:C) exhibited markedly impaired upregulation of type 1 IFN-inducible genes, including *Ifit1* and *Isg15* (Figures S1C, S1F, and S1J). We also observed that *Ptpn22* positively regulated induction of type 1 IFN-inducible genes in vivo after LPS or poly(I:C) challenge (Figure S1R). Further, TLR3- or TLR4-stimulated *Ptpn22*$^{-/-}$ BMM showed reduced upregulation of the type 1 IFN-dependent, costimulatory molecules CD86 and CD40 (Figure 1G; Figure S1S). These findings indicated that PTPN22 or Ptpn22 potentiates TLR-mediated type 1 IFN-inducible gene upregulation and cellular activation in vitro and in vivo.

Ptpn22 Promotes Optimal Antigen-Specific CD8$^+$ T Cell Expansion upon TLR Engagement

TLR signals might cooperate with antigen receptor and costimulatory molecule signals to promote optimal proliferation and functional differentiation of cytolytic CD8 T lymphocytes in vivo (Curtsinger et al., 2005). The TLR-induced "Signal 3" might be mediated by type 1 IFN. To assess the role of *Ptpn22* in TLR agonist-promoted T lymphocyte responses, we adoptively transferred wild-type (WT) OT-1 TCR transgenic T cells into WT or *Ptpn22*$^{-/-}$ hosts. Five days after injecting cognate antigen (ovalbumin) together with poly(I:C), we quantified reporter OT-1 T cell proliferation and Granzyme B expression. We found that OT-1 cells responding to OVA plus poly(I:C) in *Ptpn22*$^{-/-}$ mice displayed significantly reduced expansion (Figure 1H) and impaired induction of Granzyme B expression (Figure 1I), compared to OT-1 cells transferred into OVA plus poly(I:C)-treated control animals. Ptpn22 was not required for the modest OT-1 proliferation stimulated by antigen alone, nor was Ptpn22 required for antigen processing and presentation in vivo (Figure S2A). *Ptpn22*$^{-/-}$ mice also displayed normal splenic DC subsets compared to WT mice (Figure S2B). These findings indicated that *Ptpn22* regulates a T cell-extrinsic effect of poly(I:C) on expansion of CD8$^+$ T cells responding to antigen.

Ptpn22 Promotes Host Antiviral Responses

As type 1 IFNs are critical mediators of antiviral host defense (Stetson and Medzhitov, 2006), we investigated a possible role for Ptpn22 in antiviral immunity. Protective innate and adaptive immune responses against lymphochoriomeningitis virus (LCMV) and encephalomyocarditis virus (EMCV) depend upon type 1 IFN upregulation by pattern recognition receptors, including TLRs and intracellular RNA sensors such as MDA5 (Gitlin et al., 2006; Jung et al., 2008; McCartney et al., 2011; Zhou et al., 2010). We found that one day after LCMV exposure, *Ptpn22*$^{-/-}$ mice displayed reduced infection-induced serum IFN-α and IFN-β concentrations (Figure 2A) and blunted activation of splenic CD8α$^+$ DCs and plasmacytoid DCs (pDCs) (Figure 2B; Figures S2C and S2D). pDCs are the primary producers of type I IFNs during acute LCMV infection (Jung et al., 2008). We observed significantly fewer splenic IFN-α-producing pDCs after LCMV infection in *Ptpn22*$^{-/-}$ mice compared to WT mice (Figures 2C and 2D). However, the percentage of TNF-producing pDCs after LCMV infection was unaffected in *Ptpn22*$^{-/-}$ mice (Figures S2E and S2F). Moreover, *Ptpn22*$^{-/-}$ mice exhibited impaired expansion of LCMV-specific CD8$^+$ T cells (Figures 2E and 2F). Finally, we observed increased mortality and viral titers in *Ptpn22*$^{-/-}$ mice infected with EMCV (Figures S2G and S2H). Taken together, these data indicated that Ptpn22 promotes innate immune cell activation and cytotoxic T lymphocyte proliferation, as well as viral clearance and protection from death after infection by select viruses.

Ptpn22 Promotes poly(I:C)-Mediated Suppression of Arthritis

Mounting evidence implicates TLR signals and type 1 IFNs in immunoregulatory and anti-inflammatory processes (González-Navajas et al., 2012). To investigate the role of Ptpn22 in poly(I:C)-driven immunoregulation, we utilized a model of IL-1β-dependent inflammatory arthritis in which poly(I:C) treatment ameliorates disease in a type 1 IFN-driven manner (Ji et al., 2002; Yarilina et al., 2007). In the absence of poly(I:C), WT and *Ptpn22*$^{-/-}$ mice developed polyarthritis of equal severity after injection of arthritogenic serum (Figure 3A). WT mice exhibited dramatic reduction of serum-induced arthritis after repeated poly(I:C) treatment. In contrast, *Ptpn22*$^{-/-}$ mice exhibited robust arthritis despite poly(I:C) treatment (Figure 3A). More severe arthritis in poly(I:C)-treated *Ptpn22*$^{-/-}$ mice correlated with increased synovial leukocyte infiltration (Figure 3B). We also observed reduced serum type 1 IFN amounts, and lower synovial expression of type 1 IFN-inducible gene *Isg15*, in *Ptpn22*$^{-/-}$ mice receiving poly(I:C) treatment (Figures 3C and 3D). However, poly(I:C) treatment of WT mice resulted in a lower expression of synovial *Il1b* than was observed in *Ptpn22*$^{-/-}$ mice (Figure 3D). In serum transfer arthritis, poly(I:C) treatment suppresses

synovial IL-1β expression, and the suppressive effects of poly(I:C) are type 1 IFN-dependent (Corr et al., 2009; Yarilina et al., 2007). Therefore, the synovial *Il1b* findings suggested that *Ptpn22* might inhibit synovial inflammation in serum transfer arthritis by promoting type 1 IFN-dependent downregulation of proinflammatory cytokines.

PTPN22 regulates signaling in T and B lymphocytes (Rhee and Veillette, 2012). To ask whether adaptive immune cells are required for *Ptpn22*-dependent, poly(I:C)-induced arthritis suppression, we utilized T and B cell-deficient $Rag1^{-/-}$ mice. $Rag1^{-/-}$ mice developed robust arthritis after serum transfer (Figures 3E and 3F), as previously reported (Korganow et al., 1999). Importantly, $Rag1^{-/-}$ mice also showed poly(I:C)-driven amelioration of arthritis, equivalent to that observed in WT animals (Figures 3E and 3F). Together, these data indicated that Ptpn22 promotes poly(I:C)-driven, type 1 IFN-mediated suppression of inflammatory arthritis, and that adaptive immune cells are not required to mediate Ptpn22-dependent effects.

Ptpn22 Promotes Mucosal Homeostasis in DSS-Induced Colitis

Myeloid cell-produced type 1 IFNs protect intestinal epithelial cells against mucosal injury (Katakura et al., 2005) in the dextran sodium sulfate (DSS) mouse model of colitis (Okayasu et al., 1990). In comparison with WT littermates, DSS-exposed $Ptpn22^{-/-}$ mice developed more severe colitis, as evidenced by accelerated loss of body weight (Figure 4A) and diminished colon length (Figure 4F; Figure S3). Histological examination revealed more frequent ulceration and more extensive intramural leukocyte infiltration in colons from $Ptpn22^{-/-}$ mice (Figure 4B). Further, $Ptpn22^{-/-}$ mice exhibited reduced *Mx1* but augmented *Tnf* and *Il1b* mRNA (Figure 4C) in colonic mucosa. We also asked whether Ptpn22 potentiates TLR9-driven, DC-mediated, type 1 IFN-dependent protection of the colonic mucosa (Katakura et al., 2005). After serial CpG injection concomitant with DSS exposure, control animals showed significant protection against weight loss, colonic ulceration, and colon shortening. $Ptpn22^{-/-}$ mice displayed reduced protection after CpG treatment (Figures 4D and 4F; Figure S3). Increased mucosal ulceration in the colons of CpG-treated, DSS-exposed $Ptpn22^{-/-}$ mice again correlated with markedly reduced expression of the type 1 IFN-inducible gene *Mx1* and with upregulated *Tnf* and *Il1b* (Figure 4G). Thus, Ptpn22 promotes both constitutive type 1 IFN-dependent mucosal protection, and TLR-induced, myeloid cell-mediated, type 1 IFN-dependent amelioration of intestinal injury in DSS irritant colitis.

Ptpn22 Positively Regulates TLR-Triggered IRF3 Activation

We addressed the molecular mechanism for selective promotion of TLR-induced type 1 IFN by Ptpn22. We stimulated *Ptpn22*-deficient myeloid cells with TLR ligands and analyzed cell lysates for activation of IRF3 and of the NF-κB pathway (Sato et al., 2003). After LPS or poly(I:C) stimulation, we found reduced phosphorylation of IRF3 in $Ptpn22^{-/-}$ BMM (Figures 5A and 5B). However, we observed no change in the kinetics of LPS-induced IκBα degradation in $Ptpn22^{-/-}$ BMM (Figure S4A). Notably, after TLR7 agonist PolyU stimulation, we found diminished IRF7 nuclear translocation in $Ptpn22^{-/-}$ Flt3L-BMDCs (Figure S4B). These data indicated that Ptpn22 selectively regulates IRF3 and IRF7, but not NF-κB, activation after TLR stimulation.

Type 1 IFNs signal through IFNAR to activate signal transducer and activator of transcription-1 (STAT1) (Stetson and Medzhitov, 2006). In LPS- or poly(I:C)-stimulated $Ptpn22^{-/-}$ BMM, we observed marked reduction in STAT1 phosphorylation (Figure 5C). However, treatment with recombinant IFN-α induced normal STAT1 phosphorylation (Figure 5D), and IFN-β treatment restored LPS-stimulated *Isg15* and *Mx1* expression, in $Ptpn22^{-/-}$ BMM (Figure 5E). These data indicated that *Ptpn22* is dispensable for signaling

downstream of IFNAR. Together, the findings strongly suggested that defective type 1 IFN production, and not impaired IFNAR signaling, underlies reduced type 1 IFN-dependent effects in *Ptpn22*$^{-/-}$ cells and mice.

Ptpn22 Phosphatase Activity Is Not Required for TLR-Triggered IRF3 Activation

PTPN22 tyrosine phosphatase activity is required for negative regulation of antigen-receptor signaling (Cloutier and Veillette, 1999). To test the role of PTPN22 enzymatic activity in TLR signaling, we studied PTPN22 C227S, a phosphatase-inactive PTPN22 mutant (Cloutier and Veillette, 1999; Fiorillo et al., 2010). We found that either WT or PTPN22 C227S expression in *Ptpn22*$^{-/-}$ BMM resulted in comparable augmented type 1 IFN induction (Figure 5F). Moreover, we found no defects in either IFN-β production by TLR4-stimulated RAW 264.7 cells treated with specific Ptpn22 phosphatase inhibitors I-C11 and 4e (Stanford et al., 2011; Yu et al., 2007) (Figure S4C) or in IRF3 phosphorylation or type 1 IFN induction by LPS-stimulated BMM that were pretreated with the specific inhibitor 4g (Stanford et al., 2011) (Figures S4D and S4E). Together, these results strongly suggested that Ptpn22 enzymatic activity is dispensable for TLR signaling to type 1 IFN promoters.

Ptpn22 Interacts with TRAF3 and Positively Regulates TRAF3 K63-Linked Polyubiquitination

We speculated that PTPN22 might selectively promote TLR signaling through protein-protein association. We found that PTPN22 coimmunoprecipitates with the E3 ubiquitin ligase TRAF3, a critical mediator of TLR-induced type 1 IFN production (Häcker et al., 2011), from human peripheral blood mononuclear cell (PBMC)-derived dendritic cells (Figures 6A and 6B). The PTPN22-TRAF3 association is likely direct, because purified PTPN22 and TRAF3 specifically interact in vitro (Figure 6C). Further, the PTPN22-TRAF3 association depends upon the TRAF domain of TRAF3 but is independent of the TRAF3 RING domain that mediates E3 ubiquitin ligase function (Figures S5A and S5B). Notably, we observed no interaction between PTPN22 and either TRAF2 or TRAF6, key mediators in TNFR signaling and MyD88-dependent TLR signaling, respectively (Cabal-Hierro and Lazo, 2012; Chen, 2012) (FiguresS5C and S5D).

After TLR stimulation, K63-linked polyubiquitination of TRAF3 is required for downstream signals leading to production of type 1 IFNs (Tseng et al., 2010). We therefore investigated a possible role for Ptpn22 in TRAF3 polyubiquitination after TLR stimulation. We observed marked reduction in K63-linked polyubiquitination of TRAF3 from KLA-stimulated Ptpn22 ASO-treated RAW 264.7 cells (Figure 6D) and from LPS-stimulated *Ptpn22*$^{-/-}$ BMM (Figure 6E). TRAF3 is required for suppression of the noncanonical NF-κB pathway and loss of TRAF3 results in constitutive processing of p100 to p52 (He et al., 2006; Zarnegar et al., 2008). Interestingly, in *Ptpn22*$^{-/-}$ BMM, we found no evidence of p100 cleavage, similar to WT BMM (Figure S5E). Together, our data thus suggested that although *Ptpn22* specifically interacts with TRAF3 and positively regulates TLR-induced K63-linked polyubiquitination of TRAF3 in myeloid cells, Ptpn22 is dispensable for TRAF3-mediated suppression of the noncanonical NF-κB pathway.

We hypothesized that the human disease-associated variant PTPN22W might differentially regulate TLR signaling, compared with the common variant PTPN22R. In 293T cells, we found that coexpressed PTPN22R promoted TRAF3 K63-linked poly-ubiquitination, whereas coexpressed PTPN22W did not (Figure 6F). Notably, PTPN22W showed reduced interaction with TRAF3 compared to PTPN22R in 293T cells (Figure S5F). These data suggested that variant PTPN22W functions as a hypomorph in promotion of TRAF3 K63-linked polyubiquitination.

Autoimmunity-Associated PTPN22W Fails to Promote Type 1 IFN Induction

To investigate the possibility that PTPN22W could differentially regulate myeloid signaling, we generated mice transgenic (Tg) for human PTPN22R (*Tg620R*) or PTPN22W (*Tg620W*) regulons. The transgenic mice were interbred with *Ptpn22*$^{-/-}$ animals. Equivalent amounts of human *PTPN22* mRNA in hematopoietic cells, and comparable amounts of human PTPN22R and PTPN22W in splenocytes and dendritic cells, were observed in *Ptpn22*$^{+/-}$*Tg620R* and *Ptpn22*$^{+/-}$*Tg620W* mice (Figures S6A–S6D). In addition, we found comparable amounts of *PTPN22* and *Ptpn22* mRNA in spleen from *Ptpn22*$^{+/-}$*Tg620R* mice (not shown). These findings suggested that the tissue specificity and expression of transgenic *PTPN22* parallel those of endogenous *Ptpn22* in the mice.

We compared innate immune cell functions in *Tg620R* and *Tg620W* mice that carry either one or two *Ptpn22* null alleles. We found that, after LPS stimulation, BMDCs from *Ptpn22*$^{-/-}$*Tg620R* mice exhibited increased *Ifnb1* expression compared to BMDCs from *Ptpn22*$^{-/-}$*Tg620W* mice (Figure 7A). Similarly, BMM from *Ptpn22*$^{+/-}$*Tg620R* mice exhibited augmented LPS-stimulated upregulation of *Ifnb1* and *Ifna4* compared to BMM from *Ptpn22*$^{+/-}$*Tg620W* animals (Figure S6E). In addition, we found that upregulation of activation markers CD80 and CD40 was augmented by LPS stimulation in *Ptpn22*$^{+/-}$*Tg620R* BMM, but not in *Ptpn22*$^{+/-}$*Tg620W* BMM (Figure S6F). Collectively, these data suggested that compared to PTPN22R, PTPN22W fails to promote TLR-driven type 1 IFN upregulation and type 1 IFN-driven myeloid cell activation.

We examined the role of PTPN22W in type 1 IFN-dependent modulation of inflammatory arthritis (see Figure 3A). We found that after receiving arthritogenic serum, both *Ptpn22*$^{-/-}$*Tg620R* and *Ptpn22*$^{-/-}$*Tg620W* mice developed arthritis of comparable severity. *Ptpn22*$^{-/-}$*Tg620R* mice showed amelioration of arthritis when injected repeatedly with poly(I:C). In contrast, *Ptpn22*$^{-/-}$*Tg620W* mice exhibited persistent arthritis despite poly(I:C) treatment (Figure 7B). Compared with *Ptpn22*$^{-/-}$*Tg620R* animals, increased leukocyte infiltration was observed in the synovium of *Ptpn22*$^{-/-}$*Tg620W* mice receiving poly(I:C) treatment (Figure 7C). Moreover, we found reduced serum type 1 IFN concentrations and synovial *Isg15* expression, but increased synovial *Il1b* expression in *Ptpn22*$^{-/-}$*Tg620W* mice compared to *Ptpn22*$^{-/-}$*Tg620R* animals after poly(I:C) treatment (Figures 7D and 7E). Taken together, the data suggested that PTPN22R, but not PTPN22W, can promote poly(I:C)-driven suppression of inflammatory arthritis.

PTPN22W Carriers Show Reduced Type 1 IFN Response to LPS

We sought evidence that PTPN22W differentially regulates TLR signaling in human primary cells. We compared LPS-stimulated PBMC from healthy donors who were either carriers of the *PTPN22* risk variant (*PTPN22R/PTPN22W*; “R/W”) or age-matched noncarriers (“R/R”). We found no differences in PTPN22 amounts in PBMC-derived DCs (PBMC-DCs) or PBMC obtained from PTPN22W carriers and noncarriers (Figure S6G; data not shown). However, we observed significantly reduced STAT1 phosphorylation in PBMC from PTPN22W carriers after LPS stimulation (Figure S6H). Moreover, gene expression analysis revealed that, compared with cells from noncarriers, upregulation of type 1 IFN-inducible genes was significantly reduced in PBMC-DCs from PTPN22W carriers (Figures 7F). In contrast, LPS-induced expression of proinflammatory cytokines was similar in PBMC-DCs from PTPN22W carriers and noncarriers (Figures 7F; Figure S6I). Taken together, these findings strongly suggested that primary cells from PTPN22W carriers have a selective defect in TLR-induced type 1 IFN-dependent signaling and gene expression.

DISCUSSION

Here, we establish that *Ptpn22* connects microbial pattern sensing by innate immune cells with the induction of host defense and immunoregulatory responses mediated by type 1 IFNs. Our findings indicate that Ptpn22 is critical for efficient up-regulation of type 1 IFNs after TLR ligation in myeloid cells; that Ptpn22 promotes type 1 IFN-dependent processes, including myeloid cell activation in response to viral infection, maintenance of gut homeostasis, and suppression of inflammatory arthritis in vivo; and that Ptpn22 binds to, and potentiates K63-linked polyubiquitination of, TRAF3, a central promoter of TLR signaling leading to upregulation of type 1 IFNs. Remarkably, the human disease-associated variant PTPN22W exhibits reduced function in the promotion of TRAF3 K63-linked polyubiquitination and of type 1 IFN production in response to TLR signals.

Zhang et al. reported on myeloid cell functions in mice harboring a murine Ptpn22 ("619W") variant that is orthologous to human PTPN22W (Zhang et al., 2011). In 619W knockin DCs, Zhang et al. found augmented activation marker upregulation after LPS stimulation and increased capacity to activate antigen-specific T cells in vitro. In addition, Zhang et al. noted reduced stability of the 619W variant. In contrast to Zhang et al., our findings indicate that *Ptpn22* deficiency results in diminished TLR-dependent myeloid cell activation, both in vitro and in vivo. Notably, we found no alteration in the ability of *Ptpn22*$^{-/-}$ DCs to take up, process, and present antigen in the absence of a TLR stimulus in vivo. Incomplete degradation and/or unknown biochemical features of the 619W variant studied by Zhang et al. might explain why 619W does not phenocopy the effects of *Ptpn22* deficiency.

Zhang et al. also described enhanced proteasome-mediated degradation of the human PTPN22W variant in T cells. In contrast with Zhang et al., but in agreement with a recent report (Vang et al., 2012), we found no alteration in stability of the PTPN22W variant expressed in either murine or human cells. Differences in biochemical behavior between human PTPN22W and the mouse Ptpn22 619W variant, and/or in cell-type-specific variations in protein stability, might underlie the discrepancies between our findings and those reported by Zhang et al. Additional variation in experimental results might arise from differences in PTPN22 detection antibodies used. Because we find that PTPN22W expression is associated with reduced TLR signaling and myeloid cellular activation, parallel to findings observed with *Ptpn22* deficiency, the PTPN22W variant might be viewed as a hypomorph with respect to these functions.

Our data lend support to an emerging model wherein TRAF3 promotes TLR-induced type 1 IFNs and proinflammatory cytokines through distinct mechanisms (Häcker et al., 2011). PTPN22 positively regulates TLR-induced type 1 IFN production dependent upon nondegradative K63-linked polyubiquitination of TRAF3 and upon IRF3 activation (Tseng et al., 2010). By contrast, PTPN22 is dispensable for TLR-induced upregulation of proinflammatory cytokines, which requires TRAF3 degradation via K48-linked ubiquitination to activate NF-κB. Further analysis of the TRAF3-PTPN22 interaction will be required to understand how PTPN22 selectively potentiates type 1 IFN-inducing TRAF3 function in a PTPN22 phosphatase activity-independent manner. Possibly, PTPN22 recruits additional TRAF3-activating proteins. Impaired recruitment of such effectors arising from reduced PTPN22W association with TRAF3 could contribute to the observed defects in PTPN22W capacity to promote TRAF3 functions.

Our findings raise the intriguing possibility that enhanced susceptibility to disease in PTPN22W carriers could result from defective type 1 IFN-mediated host resistance to infection. For example, the PTPN22W-associated syndrome of Type 1 Diabetes (T1D) has

long been associated with viral infection (Boettler and von Herrath, 2011). Loss of TLR3, a type 1 IFN-inducing sensor for viral double-stranded RNA (dsRNA), renders animals susceptible to rapid T1D onset after infection with β cell-tropic EMCV (McCartney et al., 2011). In addition, islet expression of IFNAR signaling inhibitor suppressor of cytokine signaling 1 (SOCS1) potentiates Coxsackie B virus-induced T1D (Flodström et al., 2002). Together with our data showing that *Ptpn22* positively regulates TLR signaling and promotes type 1 IFN-driven antiviral responses, these observations suggest that reduced function variant PTPN22W could result in diminished human host-protective responses to diabetogenic viral infection.

Positive regulation of interferogenic TLR signals by Ptpn22 also suggests that Ptpn22 could regulate innate immunity to bacterial pathogens. Consistent with this idea, PTPN22W carriage in humans is associated with increased risk of invasive infections with the Gram-positive bacterium *S. pneumoniae* (Chapman et al., 2006). Induction of type 1 IFNs and type 1 IFN-dependent genes in alveolar macrophages by *S. pneumoniae* contributes to the clearance of bacteria (Koppe et al., 2012). Intriguing data also suggest a role for TLR-induced type 1 IFNs in innate immune responses to Gram-negative bacteria (Rathinam et al., 2012). These observations suggest that an evolutionary advantage conferred by PTPN22 might extend beyond avoiding excessive activation of T and B lymphocytes, to the promotion of protective innate immune responses against a range of infectious agents.

Type 1 IFNs play complex roles in inflammatory and autoimmune diseases (González-Navajas et al., 2012; Kalliolias and Ivashkiv, 2010). In some autoimmune syndromes (e.g., systemic lupus erythematosus [SLE]), type 1 IFNs might accelerate tissue injury (Niewold, 2011). However, type 1 IFNs can also exert potent anti-inflammatory effects; these have been observed in colitis, multiple sclerosis, autoimmune diabetes, inflammatory arthritis, and in some models of SLE (Hron and Peng, 2004; Katakura et al., 2005; Rother et al., 2009; Sanford and Lyseng-Williamson, 2011; Sobel and Ahvazi, 1998; Wu and Peng, 2006). Our data indicate that *Ptpn22* promotes inflammation suppression in models of colitis and synovitis wherein type 1 IFN-inducing TLR agonists can ameliorate tissue injury (Katakura et al., 2005; Yarilina et al., 2007). Mechanisms whereby type 1 IFNs can suppress function of key cytokines such as IL-1β in inflammatory states include downregulation of IL-1R expression, upregulation of IL-1R antagonist, and blockade of IL-1 production (Kalliolias and Ivashkiv, 2010). Our finding that PTPN22W is a reduced function variant suggests a general mechanism whereby human carriers could manifest increased inflammation in response to autoimmune or infectious stimuli.

Together, our findings document that *PTPN22* positively regulates a TLR-triggered, type 1 IFN-inducing pathway in myeloid cells and that variant PTPN22W exhibits reduced function in this pathway. Previous work has shown that Ptpn22 also serves as a regulator of proximal lymphocyte antigen-receptor signaling (Rhee and Veillette, 2012). The relative contributions of PTPN22 function in innate or adaptive immune responses to the pathology associated with human syndromes such as rheumatoid arthritis, T1D, and thyroiditis remain to be established.

EXPERIMENTAL PROCEDURES

Mice

Ptpn22$^{-/-}$ mice, crossed >10 times to C57BL/6 strain, have been described (Hasegawa et al., 2004). All mice were maintained under specific pathogen-free conditions and all studies were conducted in accordance with Institutional Animal Care and Use Committee-approved protocols at the University of Minnesota (Minneapolis, MN) and the La Jolla Institute for Allergy & Immunology (LIAI, La Jolla, CA).

Subjects

Frozen healthy donor PBMC for this study were obtained from the study, Risk Factors for the Severity of Infectious Mononucleosis Due to Primary Epstein-Barr Virus Infection in University Students (Mono 5), which was approved by the University of Minnesota Institutional Review Board (0608M90593).

LCMV Infection

Mice were injected intraperitoneally (i.p.) with 2×10^5 plaque-forming units (PFU) LCMV Armstrong. IFN-α and IFN-β were measured in sera 24 hr after LCMV infection by ELISA (PBL Biomedical Laboratories). Splenic DC activation was detected at 24 hr after LCMV exposure by FACS analysis. Intracellular IFN-α or TNF staining of splenocytes was performed after a 3 hr incubation with brefeldin A (eBioscience). Cells were stained with DC markers, followed by fixation with Cytofix/Cytoperm buffer (BD). Subsequently, cells were permeabilized by using Perm/Wash buffer (BD) and incubated with anti-IFN-α (clone RMMA-1, PBL) or anti-TNF (eBioscience). To enumerate LCMV-specific T lymphocytes, we harvested splenocytes 7 days after infection and stained with T-select H-2Db LCMV tetramer-KAVYNFATC (Beckman Coulter). Samples were acquired on a LSR II flow cytometer (BD Biosciences) and analyzed with FACSDiva software.

Serum-Transfer System and Evaluation of Arthritis

Serum-transferred arthritis was induced by i.p. injection, on days 0 and 2, of 150 μl of pooled serum obtained from adult K/BxN mice. Poly(I:C) (150 μg) or saline (150 μl) were injected i.p. every other day starting one day after serum injection. Ankle thickness was measured with a caliper (J15 flat feeler). Each limb was scored on a scale from 0 to 3.

DSS-Induced Colitis

DSS (molecular weight, 36–50 kDa; MP Biochemicals) was dissolved in water to provide a working solution of 3% (weight/volume). Mice were given free access to this solution for 10 days to induce acute colitis. Mice were weighed daily. On day 10, or when mice lost more than 20% of the initial body mass, colons were removed and measured, photographed and prepared for histology. Colons used for RNA analyses were obtained on day 3 after DSS exposure or at day 7 in experiments examining the effects of CpG on colitis.

PBMC and PBMC-DC Stimulation

Previously frozen PBMC from healthy subjects were thawed and resuspended at 1×10^6 cells/ml in RPMI-1640 with 10% FBS. Cells were cultured for 1 hr at 37°C before stimulated with LPS (1 μg/ml) for 4 hr. PBMC-DCs were resuspended at 1×10^6 cells/ml in RPMI-1640 with 10% FBS and stimulated with LPS (1 μg/ml) for 4 hr. Cells were then harvested for gene expression and immunoblotting analysis.

Real-Time Quantitative PCR

RNA was isolated using TRIzol (Invitrogen), and cDNA was synthesized using iScript (Bio-Rad), according to the manufacturer's instructions. Real-time PCR was performed as described in the Supplemental Experimental Procedures.

Immunoblotting and Immunoprecipitation

Immunoblotting and immunoprecipitation were performed as described in the Supplemental Experimental Procedures.

Statistical Analysis

Data are presented as mean ± SEM. Multiple group comparisons were done by one-way analysis of difference (ANOVA) with a Bonferroni multiple-comparison posttest. Two-tailed Student's t test was used to compare two independent groups. For all tests, a p value less than 0.05 was considered statistically significant. Prism GraphPad software v5.0 was used for statistical analyses.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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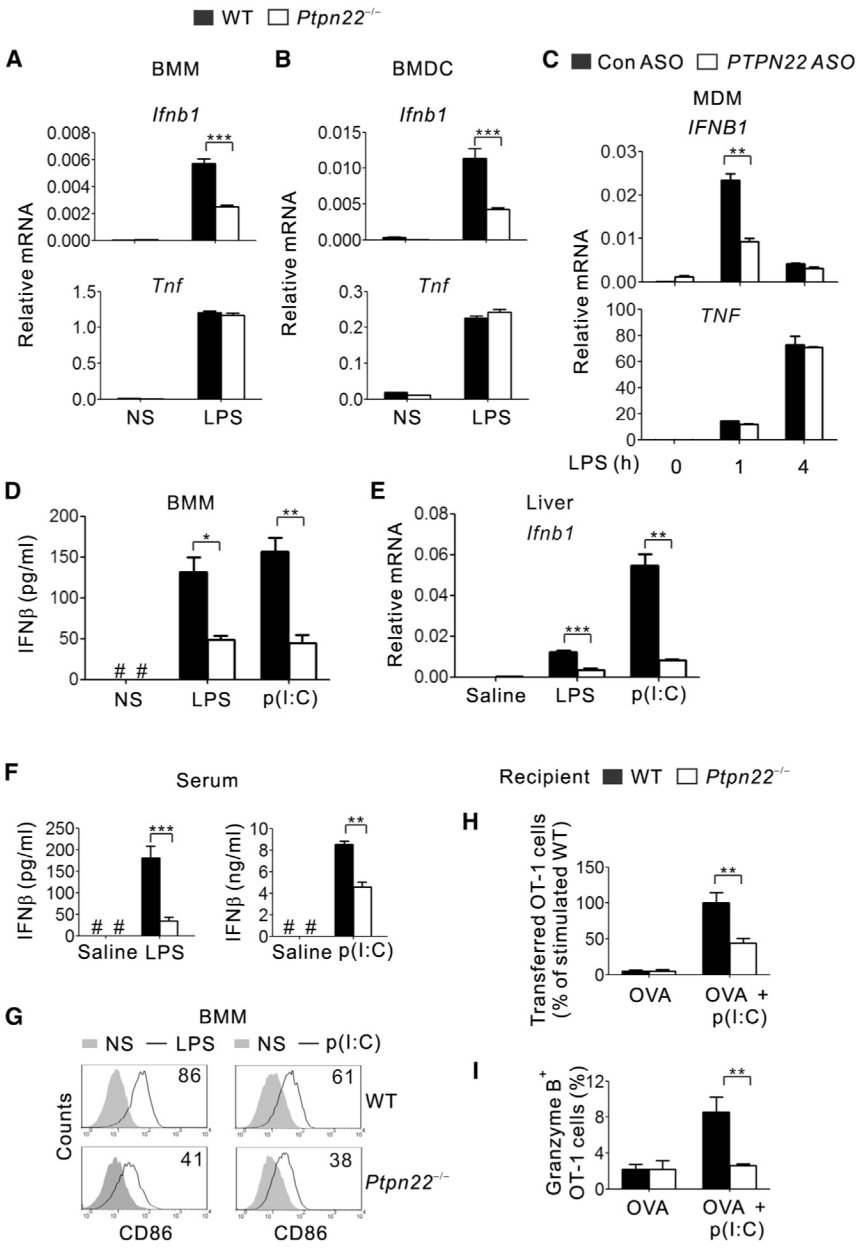

Figure 1. Ptpn22 Selectively Regulates TLR-Induced Type 1 IFN Production

(A and B) WT and Ptpn22-deficient (*Ptpn22*$^{-/-}$) BMM (A) or BMDCs (B) were stimulated with LPS (100 ng/ml) for 4 hr. Gene expression values relative to *Gapdh* were determined by qPCR on total RNA. (C) Human MDM were treated with Control (Con) or *PTPN22* ASO for 48 hr. Cells were then stimulated with LPS (100 ng/ml) for 1 and 4 hr. Gene expression values relative to *RPL13A* were determined by qPCR on total RNA.

(D) BMM were stimulated with LPS or poly(I:C) (p(I:C); 10 μg/ml) for 16 hr. IFN-β concentrations in culture supernatant were quantified by ELISA. NS, no stimulus. #, undetectable.

(E) qPCR on total liver RNA 4 hr after i.p. injection of LPS (5 mg/kg) or p(I:C) (10 mg/kg) (n = 4 per genotype).

(F) Serum IFN-β concentrations (mean ± SEM) were measured by ELISA at 1 hr after i.p. injection of LPS (left panel) or 4 hr after i.p. injection of p(I:C) (right panel).
(G) BMM stimulated with LPS or p(I:C) for 16 hr. Mean fluorescence intensity (MFI; obtained by FACS) values for LPS stimulated conditions are shown.
(H and I) WT OT-1 Tg $CD8^+$ T cells (1×10^5) were adoptively transferred into hosts of indicated genotype. Mice were immunized with ovalbumin (OVA) alone or with OVA plus p(I:C) (n > or = 7 per genotype). On day 5, the numbers (H) and fractions of Granzyme B^+ (I) OT-1 T cells in draining lymph nodes were detected by FACS. Data shown are mean ± SEM (H and I). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Data are representative of two (C) or three (A and B, and D–F) independent experiments with similar results (mean ± SEM in A–E). See also Figure S1.

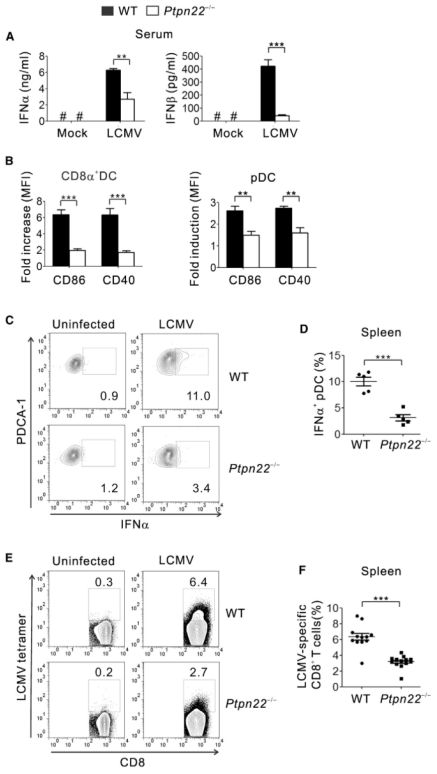

Figure 2. Ptpn22 Promotes Host Antiviral Responses In Vivo

(A–F) Mice were i.p. inoculated with LCMV (2×10^5 PFU). (A) Serum IFN-α and IFN-β concentrations were measured by ELISA at 24 hr after infection. (B) Upregulation of CD86 and CD40 on splenic CD8α$^+$ DCs (CD8α$^+$CD11c$^+$) or plasmacytoid DCs (pDCs; B220$^+$PDCA1$^+$) was assayed by FACS at 24 hr after infection. Fold increases in MFI (mean ± SEM) are shown (n = 6 per genotype, (A) and (B). (C and D) IFN-α expression in splenic pDCs at 24 hr after LCMV infection was detected by intracellular staining. (C) Numbers shown on representative plots are percentages of pDCs staining positive for IFN-α. (D) Percentages of IFNα$^+$ pDCs in individual animals; means ± SEM are indicated (n = 5 per genotype). (E and F) Percentages of LCMV tetramer$^+$ CD8$^+$ T cells in spleen at day 7 after infection are shown (E) and means ± SEM are indicated (F) (n > or = 12 per genotype). **p < 0.01, ***p < 0.001. Data are representative of three independent experiments with similar results (mean ± SEM in A, B, D, and F). See also Figure S2.

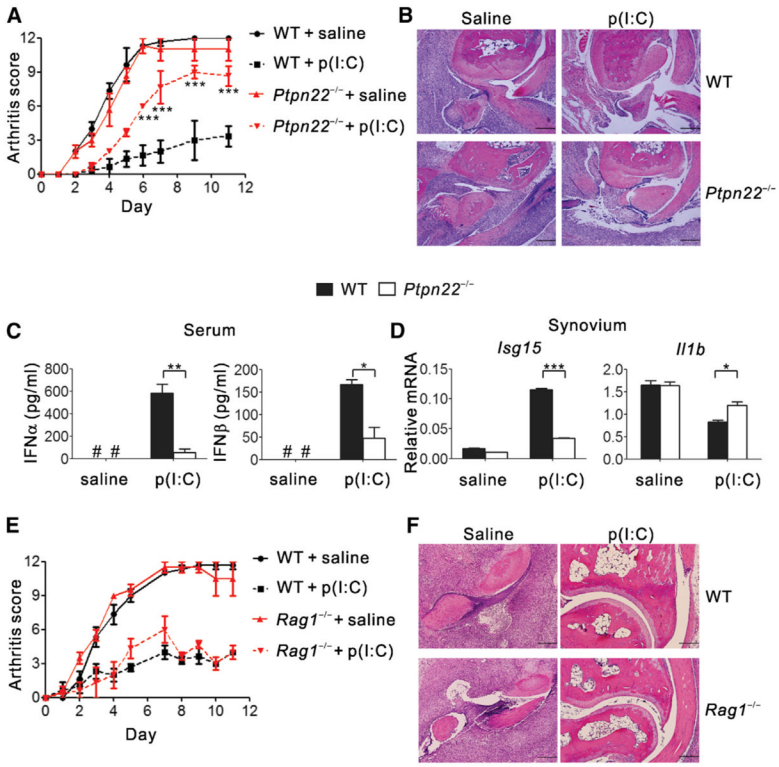

Figure 3. Ptpn22 Promotes poly(I:C)-Mediated Suppression of Inflammatory Arthritis

(A–F) Mice (n = 3 per group) were injected i.p. with K/BxN serum and were treated with vehicle or with p(I:C) i.p. (A and E) Arthritis severity scores (mean ± SEM) are shown. (B and F) Ankle joint sections were stained with hematoxylin and eosin (H&E). Scale bar represents 200 μm. (C) Serum IFN-α and IFN-β (mean ± SEM) at day 11 were measured by ELISA. (D) Gene expression (mean ± SEM) relative to *Gapdh* in mouse synovium was determined by qPCR. *p < 0.05, **p < 0.01, ***p < 0.001.

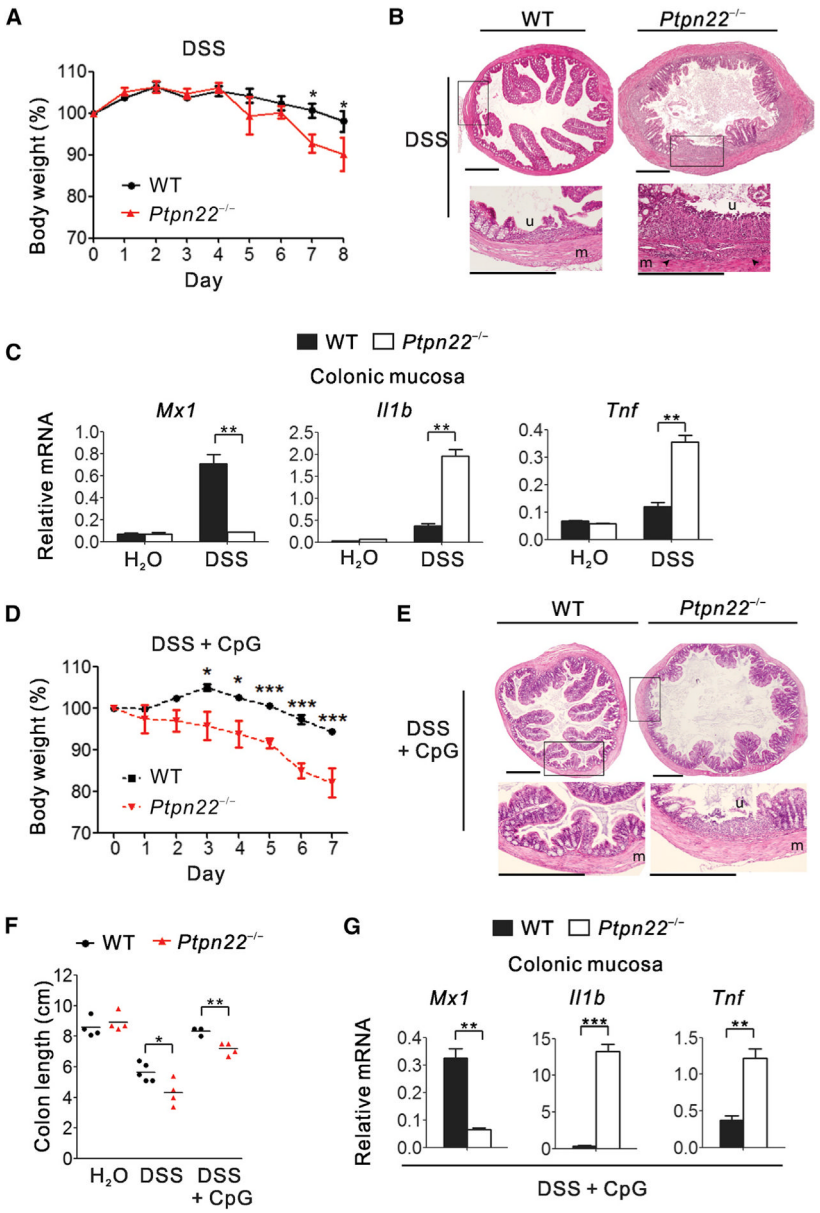

Figure 4. Ptpn22 Promotes Mucosal Homeostasis in DSS-Induced Colitis

(A–G) Mice were exposed to 3% DSS in drinking water. Some mice also received CpG (10 μg per animal) i.p. injection 3 hr before and at days 4 and 6 of DSS exposure (D–G). (A) Body weight (mean ± SEM) of WT (n = 5) and *Ptpn22*$^{-/-}$ (n = 4) mice, relative to initial body weight, on days 1–8 after exposure to 3% DSS in drinking water. (B and E) Colonic sections were prepared and stained with H&E. Colon ulceration (u), muscle hypertrophy (m) and leukocyte infiltration (arrowheads) are indicated. Scale bar represents 500 μm. (C and G) Expression of *Mx1*, *Il1b*, and *Tnf* was measured in colon harvested at days 4 (C) and 8 (G) by qPCR (mean ± SEM). Values were relative to *Rpl13*. (D) Body weight (mean ± SEM) of WT (n = 3) and *Ptpn22*$^{-/-}$ (n = 4) mice receiving CpG (10 μg per mouse) i.p. injection 3 hr prior to, and at days 4 and 6 following start of, exposure to 3% DSS. (F) Colons were harvested at day 8–10 after mice showed a > 20% drop in body weight, and

length (mean ± SEM) was measured. Each data point represents an individual colon. *p < 0.05, **p < 0.01, ***p < 0.001. See also Figure S3.

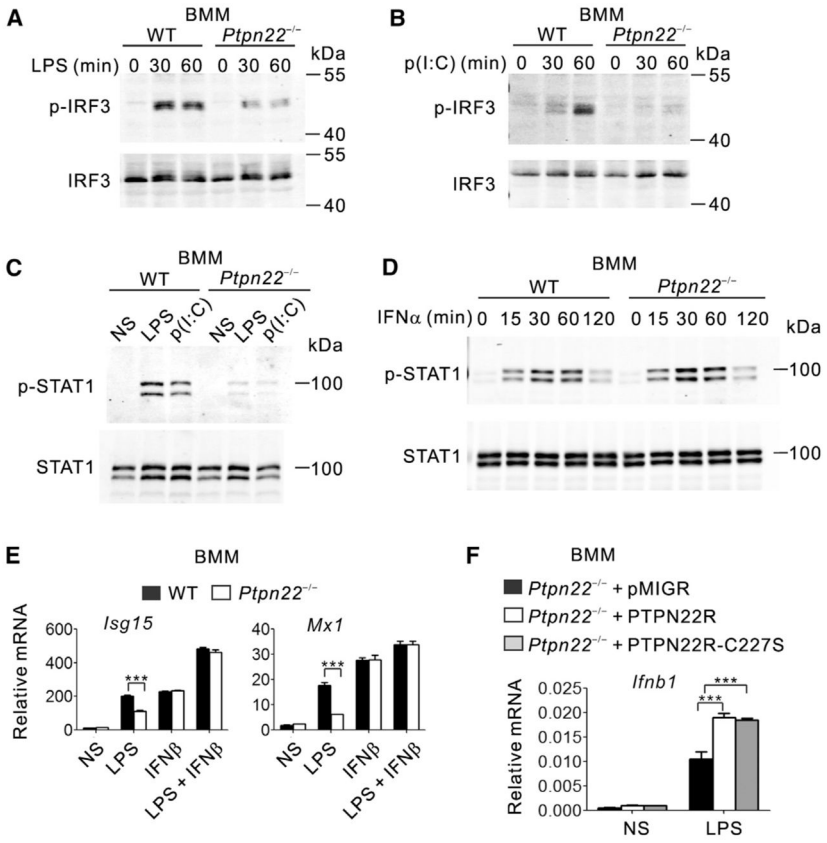

Figure 5. Ptpn22 Selectively Regulates TLR-Induced IRF3 Activation

(A and B) LPS (A) or p(I:C) (B) stimulated BMM lysates were probed with anti-phospho-IRF3 (p-IRF3) or anti-total IRF3.

(C) Lysates from BMM stimulated with LPS or p(I:C) for 4 hr were probed with anti-phospho-STAT1 (p-STAT1) and anti-total STAT1.

(D) BMM were stimulated with recombinant IFN-α (1,000 U/ml). Whole cell lysates from indicated time points were probed for phospho-STAT1 (p-STAT1) and total STAT1.

(E) Gene expression in BMM stimulated with LPS alone or with LPS plus recombinant IFNβ (10 U/ml) for 6 hr was determined by qPCR. Expression values (mean ± SEM) were relative to *Rpl13*.

(F) *Ptpn22*$^{-/-}$ BMM were infected with empty retro-virus (pMIGR), virus harboring PTPN22R, or phosphatase inactive variant PTPN22R-C227S for 48 hr. Cells were then stimulated with LPS for 4 hr and expression of *Ifnb1* was determined by qPCR. Expression values (mean ± SEM) were relative to *Gapdh*. ***p < 0.001. Data are representative of three independent experiments (A–D). See also Figure S4.

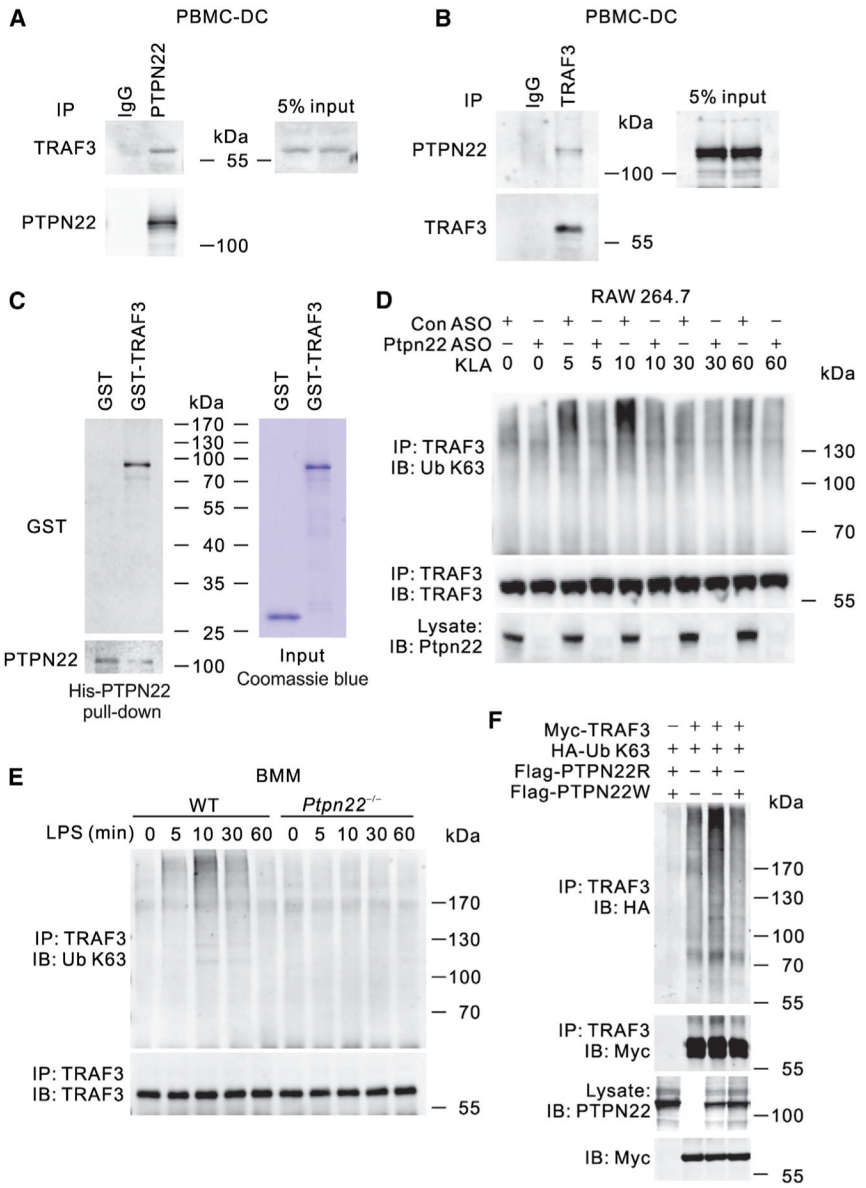

Figure 6. Ptpn22 Specifically Interacts with TRAF3 and Positively Regulates TLR-Induced K63-Linked Polyubiquitination of TRAF3

(A and B) Lysates from PBMC-derived DCs were incubated with control immunoglobulin G, anti-PTPN22 antibody (A), or anti-TRAF3 antibody (B) followed by immunoprecipitation (IP) and immunoblotting with indicated antibodies.

(C) His pulldown from mixtures of His-PTPN22R with GST or with GST-TRAF3.

(D) RAW 264.7 cells were treated with cell-permeable antisense oligonucleotide (ASO) against *Ptpn22* or with inverse *Ptpn22* sequence control (Con) ASO for 48 hr. Cells were then stimulated with 10 ng/ml Kdo_2-lipid A (KLA) and K63-linked poly- ubiquitination of TRAF3 was detected by IP and IB with indicated antibodies.

(E) K63-linked polyubiquitination of TRAF3 in WT and *Ptpn22*$^{-/-}$ BMM after LPS stimulation.

(F) IP and IB were performed on lysates from HEK293T cells transiently transfected with expression plasmids encoding indicated molecules to detect K63-linked polyubiquitination

of TRAF3. Ub, ubiquitin. Data are representative of two (D) or three (A–C, E, F) independent experiments. See also Figure S5.

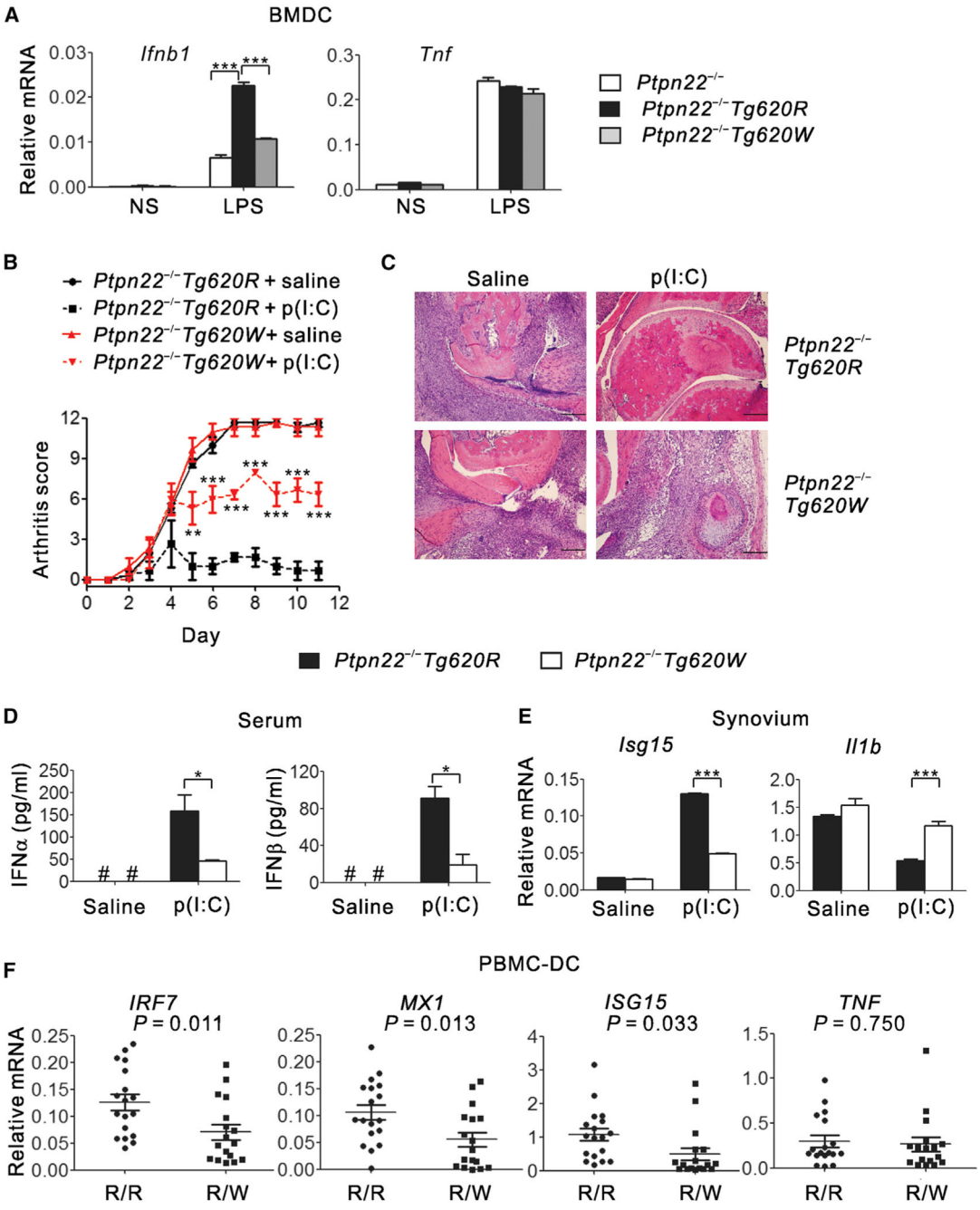

Figure 7. Disease-Associated PTPN22W Variant Exhibits Reduced Function in Promoting Type 1 Interferon Production

(A) BMDCs from indicated mice were stimulated with LPS (100 ng/ml) for 4 hr, and gene expression relative to *Gapdh* was determined by qPCR (mean ± SEM).

(B–E) Mice were injected i.p. with K/BxN serum alone or with p(I:C) i.p. (n = 3 per group). (B) Arthritis severity scores (mean ± SEM) are shown. (C) Ankle joint sections were stained with hematoxylin and eosin (H&E). Scale bar represents 200 μm. (D) Serum IFN-α and IFN-β (mean ± SEM) at day 11 were measured by ELISA. (E) Gene expression relative to *Gapdh* in mouse synovium was determined by qPCR (mean ± SEM).

(F) PBMC-DCs from healthy donors carrying PTPN22W (R/W, n = 17) and noncarriers (R/R, n = 18) were stimulated with LPS (1 μg/ml) for 4 hr and gene expression was analyzed by qPCR. Expression values (mean ± SEM) are relative to *GAPDH*. *p < 0.05, **p < 0.01, ***p < 0.001. See also Figure S6.